

CLAIMS

1. An oligonucleotide, which at least comprises, in a direction from the 5'-terminus to the 3'-terminus:

- (1) an antisense sequence of a target nucleic acid sequence;
- (2) a trimming sequence which is cleaved with base-specific RNase;
- (3) a sense sequence of a target nucleic acid sequence;
- (4) an antisense sequence of a promoter sequence;
- (5) a sequence that forms a loop; and
- (6) a sense sequence of a promoter sequence,

wherein the above-described antisense sequence and sense sequence of a promoter sequence form a double strand in a molecule via a hairpin structure, and when DNA is transcribed, a transcriptional product from the above-described antisense sequence and sense sequence of a target nucleic acid sequence forms a double strand in a molecule via the trimming sequence.

2. An oligonucleotide, which at least comprises, in a direction from the 5'-terminus to the 3'-terminus:

- (1) an antisense sequence of a target nucleic acid sequence;
- (2) a trimming sequence which is cleaved with base-specific RNase;
- (3) a sense sequence of a target nucleic acid sequence; and
- (4) an antisense sequence of a promoter sequence,

wherein, when DNA is transcribed, a transcriptional product from the above-described antisense sequence and sense sequence of a target nucleic acid sequence forms a double strand in a molecule via the trimming sequence.

3. The oligonucleotide according to claim 2 wherein at least a promoter sequence region is double-stranded.

4. A double-stranded DNA, which consists of the oligonucleotide of claim 2 and an

oligonucleotide having a sequence complementary to said oligonucleotide.

5. The oligonucleotide according to any of claims 1 to 4 which has two bases AA at the 5'-terminus located upstream of the antisense sequence of a target nucleic acid sequence.

6. The oligonucleotide according to any of claims 1 to 5 wherein the trimming sequence which is cleaved with RNase is represented by 5'-C(D)_kCD-3' wherein D represents A, T, or G, and k represents an integer between 0 and 100, wherein (k + 1) number of D bases may be identical to or different from one another.

7. The oligonucleotide according to any of claims 1 to 6 wherein the trimming sequence which is cleaved with RNase is represented by 5'-CTATGCT-3'.

8. The oligonucleotide according to any of claims 1 to 7 wherein -CCC- exists between the sense sequence of a target nucleic acid sequence described in (3) and the antisense sequence of a promoter sequence described in (4).

9. The oligonucleotide according to any of claims 1 to 8 wherein the promoter sequence is a T7 class III promoter sequence.

10. The oligonucleotide according to any of claims 1 to 9 wherein the sequence that forms a loop described in (5) is a sequence comprising -GNA- wherein N represents A, T, C, or G.

11. An oligonucleotide represented by 5'-AA-(the antisense sequence of a target nucleic acid sequence)-CTATGCT-(the sense sequence of a target nucleic acid sequence)-CCC-TATAGTGAGTCGTATTA-GCGAAGC-TAATACGACTCACTATA-3'.

12. A method for producing shRNA, which comprises transcribing DNA, using the oligonucleotide or DNA of any of claims 1 to 11 as a template and using RNA polymerase.

13. The method for producing shRNA according to claim 12 wherein the transcription is carried out *in vitro*.

14. The method for producing shRNA according to claim 12 or 13 wherein T7 RNA

polymerase is used as RNA polymerase.

15. shRNA produced by the method of any of claims 12 to 14.

16. A method for producing siRNA, which comprises treating the shRNA produced by the method of any of claims 12 to 14 with base-specific RNase.

17. A method for producing siRNA, which comprises transcribing DNA using the oligonucleotide of any of claims 1 to 11 as a template and using RNA polymerase, so as to produce shRNA, and then treating the shRNA with base-specific RNase.

18. A method for suppressing the expression of a gene containing a target nucleic acid sequence by RNAi, using the shRNA produced by the method of any of claims 12 to 14 or siRNA produced by the method of claim 16 or 17.

19. A reagent kit for carrying out the method of any of claims 12 to 14 and 16 to 18 which comprises RNA polymerase and base-specific RNase.